International Journal of Toxicology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713926068

Safety Studies Regarding a Standardized Extract of Fermented Wheat Germ

To cite this Article: Heimbach, James T., Sebestyen, Gyula, Semjen, Gabor and Kennepohl, Elke , 'Safety Studies Regarding a Standardized Extract of Fermented Wheat Germ', International Journal of Toxicology, 26:3, 253 - 259
To link to this article: DOI: 10.1080/10915810701369709
URL: http://dx.doi.org/10.1080/10915810701369709

International Journal of Toxicology, 26:253–259, 2007

ISSN: 1091-5818 print / 1092-874X online
DOI: 10.1080/10915810701369709

Safety Studies Regarding a Standardized Extract of Fermented Wheat Germ

James T. Heimbach
JHeimbach LLC, Port Royal, Virginia, USA

Gyula Sebestyen
Department of Public Health, Faculty of Medicine, Semmelweis University, Budapest, Hungary

Gabor Semjen
Department of Pharmacology and Toxicology, Faculty of Veterinary Science, Szent Istvan University, Budapest, Hungary

Elke Kennepohl
Write-Tox Consulting, Spruce Grove, Alberta, Canada

"Avemar pulvis" is a powder consisting of an aqueous extract of fermented wheat germ, with the drying aids maltodextrin and silicon dioxide, standardized to contain approximately 200 μg/g of the natural constituent 2,6-dimethoxy-*p*-benzoquinone. The results of toxicological and clinical studies of this product demonstrate its safety for its intended use as a dietary supplement ingredient in the United States. Avemar pulvis has been used in Hungary since 1998 and is approved in that country, as well as in the Czech Republic, Bulgaria, and Romania, as a "medical nutriment for cancer patients." Acute and subacute toxicity studies using rodents orally administered Avemar pulvis showed that dose levels (2000 to 3000 mg/kg body weight [bw]/day) exceeding the normal recommended oral dosage (8.5 g/day or 121 mg/kg bw/day for a 70-kg individual) by up to approximately 25-fold caused no adverse effects. The test substance showed no evidence of mutagenicity or genotoxicity in vitro or in vivo. Clinical studies using Avemar pulvis as a supplement to drug therapy in cancer patients at doses of 8.5 g/day not only showed no evidence of toxicity, but also showed a reduction in the side effects of chemotherapy. Overall, it was concluded that Avemar pulvis would not be expected to cause adverse effects under the conditions of its intended use as an ingredient in dietary supplements.

Keywords Avemar pulvis, Dietary Supplement, DMBQ, Fermented Wheat Germ, Safety, Toxicity

The dietary supplement ingredient Avemar pulvis consists of an aqueous extract of the germ of wheat (*Triticum vulgare*) fermented with baker's yeast (*Saccharomyces cerevisiae*) combined with the drying aids maltodextrin and silicon dioxide, and standardized to contain approximately 200 μg/g of the naturally occurring constituent 2,6-dimethoxy-*p*-benzoquinone (2,6-DMBQ). The process by which the 2,6-DMBQ content of fermented wheat germ extract is standardized was invented by Hungarian biochemist Mate Hidvegi in the early 1990s. It is produced by fermenting a fixed amount of food-grade wheat germ and water with a fixed amount of baker's yeast in stainless steel fermentation vessels. The ratio of these ingredients is similar to those commonly used in the preparation of whole-wheat baked products. Fermentation proceeds with continuous stirring at controlled pH, temperature, and flow of filtered air for approximately 18 h. The fermentation product is decanted, separated, and fine-filtered to a cell-free solution, which is condensed under vacuum to a specified weight percentage. Food-grade maltodextrin and colloidal silicon dioxide are added and the mixture is spray-dried. The final product, Avemar pulvis, comprises 63.2% fermented wheat germ, 35.0% maltodextrin, and 1.8% colloidal silicon dioxide. The product is manufactured under pharmaceutical good manufacturing practices (GMP) and its consistent 2,6-DMBQ content has been confirmed by analysis of multiple lots of product (Tomoskozi-Farkas and Daood 2004; Boros, Nichelatti, and Shoenfeld 2005). Flavors and sweeteners may be added to Avemar pulvis and the product sold under various trade names, including Avemar and Avé.

Wheat germ has a long history of consumption as a food. The germ is part of the wheat kernel, along with the bran and the endosperm. Like the bran, the germ is removed in the processing of wheat to yield white flour, but it is retained in whole-wheat

Received 10 January 2007; accepted 15 March 2007.
Authors Heimbach and Kennepohl received financial support for the preparation of this manuscript from American BioSciences, Inc., New York, New York, USA.
Address correspondence to James T. Heimbach, PhD, FACN, JHeimbach LLC, 923 Water Street, P.O. Box 66, Port Royal, VA 22535, USA. E-mail: jh@jheimbach.com

products. The germ constitutes approximately 2.5% (range 2% to 4%) of the total weight of the wheat kernel (Tsen 1985; Nichelatti and Hidvegi 2002). A loaf of whole-wheat bread containing about 2 cups of whole-wheat flour (~270 g) includes about 7 g of wheat germ. Additionally, wheat germ is used as an ingredient in a wide variety of foods formulated to provide advantageous nutritional characteristics. Although it is likely that the average American's current consumption of wheat germ is <1 g/day, it appears probable that many Americans—particularly those with a preference for whole grain foods—may consume >5 g/day of wheat germ. Those consuming whole wheat at the estimated 95th percentile of wheat consumption (600 g/day, based on data from the U.S. Department of Agriculture's Economic Research Service [USDA/ERS 2005]) have a daily intake of wheat germ of approximately 15 g (2.5% of 600 g). In addition to the wheat germ consumed in whole-wheat products, many Americans may ingest wheat germ marketed as a food supplement. Milled wheat germ has been sold for many years for incorporation into baked products, addition to breakfast cereals, use as a meat filler, and other applications.

Naturally occurring 2,6-DMBQ has been identified in numerous plant families in addition to wheat, including several species that are consumed as food (e.g., *Compositae* [ettuce, endive, artichoke, chicory, sunflower, safflower, etc.], *Leguminosae* [soy, beans, peas, lentils, etc.], *Proteaceae* [macadamia], *Gramineae* [cereal grains], *Ericaceae* [blueberry, huckleberry, cranberry, wintergreen]) (Handa et al. 1983). Given that some of these plant families are widely consumed, frequently in large quantities, it is likely that humans have considerably greater exposure to 2,6-DMBQ in their diets than would result from consumption of wheat germ alone.

Uses in Food

Avemar pulvis has been used in Hungary since 1998 as a dietary supplement and was approved in 2002 as a "medical nutriment for cancer patients." It is classified by the European Union (EU) as a "dietary food for special medical purposes," a category of foods specifically processed or formulated and intended for the dietary management of patients and to be used under medical supervision, and is sold with this designation in the Czech Republic, Bulgaria, and Romania. It is also sold as a dietary supplement in Italy, Serbia, Switzerland, Cyprus, Russia, Israel, Austria, Slovakia, South Korea, Taiwan, Japan, Hong Kong, Australia, and New Zealand.

In the United States, Avemar pulvis is intended to be added to dietary supplements at a level of 8.5 g/dose for an average adult. For a 70-kg individual, this is equivalent to an intake of 121 mg Avemar pulvis/kg body weight (bw)/day. Because individuals weighing more than 90 kg (200 pounds) may increase the amount consumed up to a limit of 2 doses, it is anticipated that their intake of Avemar pulvis, in mg/kg bw/day, will generally be in the same range. The highest intake would be for a 90-kg individual consuming 2 doses: 189 mg Avemar pulvis/kg bw/day.

Because the 2,6-DMBQ content of Avemar pulvis is 200 μg/g, an 8.5-g dose of Avemar pulvis contains 1.7 mg 2,6-DMBQ. This intake of 2,6-DMBQ could be supplied by eating 17 g of wheat germ (Tomoskozi-Farkas and Daood 2004) or about 700 g of whole wheat bread (Posner 1985), respectively.

TOXICOLOGICAL STUDIES

Although wheat germ is a commonly consumed food with no known adverse effects, the effects of Avemar pulvis, a product containing 63% fermented wheat germ, were investigated in various studies, mostly unpublished, using rats or mice (Hidvegi et al. 1998, 1999; Csikó, Semjen, and Ratz 1999; Lehel, Semjen, and Ratz 1999; Zalatnai et al. 2001; Szende et al. 2004). The experimental protocols were approved by the Hungarian Ethical Committee for Preventing Abuse and Torture of Animals. As described below, there were no instances of any adverse reactions.

Acute Studies

Two acute oral toxicity studies were conducted by the same laboratory using mice and rats. These studies, as well as a third acute oral toxicity study conducted by a different laboratory, were performed according to Good Laboratory Practice (GLP) guidelines and following the *Guidelines for the Testing of Chemicals* (No. 401: "Acute Oral Toxicity") promulgated by the Organization for Economic Cooperation and Development (OECD).

Groups of 6-week-old CD-1 mice (10/sex/group) were administered by gavage a single dose of 0 (vehicle control) or 2000 mg/kg bw of Avemar pulvis in distilled water and observed for up to 14 days (Lehel, Semjen, and Ratz 1999). Animals were housed 5 per cage and were given rodent diet and tap water ad libitum; intakes were not recorded. Body weights were recorded on the day of arrival, day of randomization, prior to treatment, and 24 h post dosing. Because some weight loss (not statistically significant) was noted among some animals in both the experimental and control groups at the 24-h interval (although there were no differences in the group mean weights), mice were weighed daily thereafter until an increase in body weight was noted. Animals also were weighed on days 7 and 14 post dosing. The animals were observed twice daily for any adverse clinical signs; after the observation period, the mice were killed for a postmortem examination. No deaths or abnormal clinical signs were reported. There were no statistically significant differences in body weight between control and treated mice. Pathological examination showed no macroscopic lesions. The LD_{50} of Avemar pulvis in male and female mice in this study was >2000 mg/kg bw.

In a similar study, groups of 5-week-old Wistar rats (10/sex/group) were administered by gavage a single dose of 0 (vehicle control) or 2000 mg/kg bw of Avemar pulvis in distilled water and observed for up to 14 days (Csikó, Semjen, and Ratz 1999). The rats were treated in the same manner as the mice in the previous study and the same parameters were

examined as those in the mouse study. No deaths or abnormal clinical signs were reported. There were no statistically significant differences in body weight between control and treated rats. Pathological examination showed no macroscopic lesions. The LD_{50} of Avemar pulvis in male and female rats in this study was >2000 mg/kg bw.

A third acute oral toxicity study was conducted using Wistar rats (IPCM 1999). Groups of male and female rats were administered a single gavage dose of 0 (control) or 5000 mg/kg bw of Avemar pulvis in distilled water. There were no differences between the test animals and controls in the body weights of the animals, and no cardiovascular, respiratory, neurological, or other adverse effects were reported. Based on the absence of adverse effects, the oral LD_{50} of Avemar pulvis in male and female rats in this study was >5000 mg/kg bw.

Subacute Studies

A 28-day study with a 14-day recovery period was conducted under GLP using rats (Csikó et al. 2000). The study design met the criteria outlined in OECD Guideline 407 ("Repeated Dose 28-Day Oral Toxicity Study in Rodents") and was conducted as a limit test.

Groups of 4.5-week-old Wistar BR rats (20/sex/group) were given by gavage 0 (vehicle control) or 2000 mg/kg bw/day of Avemar pulvis in 1% methylcellulose for 28 days. Half of the animals (10/sex/group) were followed for a 14-day recovery period. The animals were housed 2 per cage and were given rodent diet and tap water ad libitum. Body weights were recorded on the day of arrival, day of randomization, prior to treatment, on days 7, 14, 21, and 28 post dosing, and on days 7 and 14 of the recovery period. The rats were observed twice daily for any adverse clinical signs or mortality during the treatment and recovery periods. Feed consumption (g/day) was recorded weekly throughout the entire study and water intake (g/day) was recorded daily during weeks 1 and 4 of the treatment period and during week 2 of the recovery period. Prior to termination, fasting blood samples were taken from the femoral vein for hematological and clinical chemistry evaluations. Hematological parameters included red blood cell count, white blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, prothrombin time, and differential leucocyte count. Clinical chemistries included glucose, total protein, albumin, total bilirubin, urea nitrogen, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, cholesterol, triacylglycerols, Na, K, Ca, Cl, and P. Urine samples also were taken for urinalysis by test strip (Hemoket/GPH5) at the end of the treatment and recovery periods. Rats dying during the study and those terminated at end of the treatment and recovery periods underwent necropsy, including gross pathology and histopathological examination of the lymph nodes, mammary glands, salivary glands, sternebrae, femur (including marrow), pituitary, thymus, trachea, lungs, heart, thyroid, esophagus, stomach, small intestine, colon, liver, gall bladder, pancreas, spleen, kidneys, adrenals, bladder, prostate, testes, ovaries, uterus, brain, eyes, and spinal cord. Organ weights were recorded. Results were statistically analyzed using the *t* test.

No adverse clinical signs were reported with the exception of one treated female rat which showed mild lethargy with decreased motor activity and piloerection 1 day prior to death on the first day of the recovery period. Two other rats from the Avemar pulvis–treated group died during the treatment period. Pathological examination revealed these deaths to be gavage errors rather than inherent toxicity of the test compound. No statistically significant differences were observed in body weight, feed consumption, water intake, urinalysis, pathological examinations, organ weights, or histopathology between control and treated rats. Hematological parameters were similar between control and treated rats, with the exception of slight but statistically significant increases in hemoglobin content, MCH, and MCHC in treated females at the end of the 2-week recovery period. These small changes were considered not to be related to the test compound and of no biological importance. Clinical chemistry values remained within normal ranges, with the exception that control males had statistically significantly higher AST (124.70 ± 25.56 U/L versus 83.70 ± 16.04 U/L) and ALT (65.23 ± 7.57 U/L versus 54.73 ± 6.07 U/L) activities compared to treated males after 28 days of treatment, but these were considered to be due to moderate hemolysis of samples. Further information on the extent of sample hemolysis was not provided in the study report. Na, K, and Cl ions were significantly elevated in control animals; however, the differences were small and not considered biologically important.

Based on these findings, the no observed adverse effect level (NOAEL) in this oral subacute study was the tested dose of 2000 mg/kg bw/day of Avemar pulvis.

Subchronic Studies

Groups of F344 rats and C57BL/6 mice (13/group) were administered 0 or 3000 mg/kg bw/day of Avemar pulvis by gavage for up to 77 days (FIP 1997). Although this study was not conducted under GLP or in accordance with OECD guidelines, histological samples were extracted and prepared under Registry of Industrial Toxicology Animal-Data (RITA) guidelines (Bahnemann et al. 1995). In addition to Avemar pulvis, 900 mg vitamin C/kg bw/day was also administered to the animals by gavage. Animals were observed for any abnormal clinical signs and body weights were recorded on treatment days 1, 4, 8, 11, 17, 22, 29, 40, 52, 61, and 74 for rats and days 1, 7, 13, 18, 32, 39, 48, 61, 67, and 77 for mice. On day 77, all rats and mice were killed using anesthesia and underwent necropsy. No hematology or clinical chemistry was performed. Organ and tissue samples were taken from 3 randomly selected animals per species in both the experimental and control groups and prepared using hematoxylin and eosin staining for histopathological examination by a board-certified pathologist in a blinded setting. Organs (heart,

lung, thymus spleen, liver, kidneys, and testicles) were removed and weighed for both species from the experimental groups only; organs from the control groups were not weighed.

There were no statistically significant differences in body weight between treated animals and their corresponding controls. No pathological changes were noted in either rats or mice.

Based on these findings, the NOAEL in this oral subchronic study was the tested dose of 3000 mg/kg bw/day of Avemar pulvis.

Mutagenicity/Genotoxicity Studies

Avemar pulvis

In a study of the ability of Avemar pulvis to inhibit the development of colonic tumors, 100 4-week-old inbred male F344 rats were randomized into four groups which were treated in the following manner over a 32-week period: (A) untreated control ($n = 10$); (B) animals ($n = 48$) received three subcutaneous injections (1 week apart) of 15 mg azoxymethane/kg bw starting at week 2 to induce colon carcinogenesis; (C) animals ($n = 32$) were gavaged daily with 3000 mg Avemar pulvis/kg bw for the entire 32-week period and received three subcutaneous injections (1 week apart) of 15 mg azoxymethane/kg bw starting at week 2; and (D) animals ($n = 10$) were gavaged daily with 3000 mg Avemar pulvis/kg bw for the entire 32-week period (Zalatnai et al. 2001). Rats from all four groups were killed by exsanguination after 32 weeks. A side study, not reported by Zalatnai et al. (2001), was performed to evaluate the genotoxicity of Avemar pulvis (Csik 2000). Bone marrow was removed from the femur, and slides were prepared and microscopically examined for the number of polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs). The number of PCEs was slightly less (33%) when compared to the water control (38%), but this was not considered biologically significant. Avemar pulvis did not show genotoxic potential in this study.

As part of a larger study, Avemar pulvis was studied in *Drosophila melanogaster* (NICS 2004). Groups of flies (10/sex/group) were fed standard nutrient solutions containing 10% sucrose or 10% Avemar pulvis for 9 days. The number of deaths was recorded daily and surviving hatching flies were counted on day 13. There were no deaths and Avemar pulvis showed no toxic effects.

The same research group (NICS 2004) also investigated the effect of Avemar pulvis on results with cobalt chloride, formaldehyde, and urethane in the *Drosophila* somatic mutation and recombination tests. Flies carrying one-one recessive mutations on their third chromosome (mwh/flr) were fed nutrient mixes containing the subject chemicals with or without Avemar pulvis or sucrose. As part of this study, groups of flies were also fed 10% Avemar pulvis or 10% sucrose alone. New mutations or somatic recombinations are visible in microscopic examination of the wings of adult flies. Mutation frequency was determined by dividing the number of visible mutations by the number of flies examined. The mutation frequency of flies fed 10% Avemar pulvis alone was similar to that of controls. Avemar pulvis showed no mutagenic potential in this study.

2,6-DMBQ

Because 2,6-DMBQ is a constituent of wheat germ (100 μg/g), and consequently of Avemar pulvis (200 μg/g), the potential genetic toxicity of this substance was reviewed. When tested at concentrations of 0.1 to 100 μg/plate in *Salmonella typhimurium* strains TA98 and TA100, 2,6-DMBQ showed no significant increase in the number of revertant colonies in the presence or absence of metabolic activation (Mohtashamipur and Norpoth 1984).

In Chinese hamster ovary (CHO) cells, 2,6-DMBQ was reported to cause a dose-dependent increase in DNA fragmentation following a 1-h exposure at concentrations of 0.5 to 0.125 mM (Brambilla et al. 1986). Similarly, when Chinese hamster lung cells (V79) or hepatocytes from Sprague-Dawley rats were exposed for 1 h to 2,6-DMBQ, a dose-dependent increase in DNA fragmentation was observed (Brambilla et al. 1988). The hepatocytes were also tested at 20 h and showed no increase in DNA fragmentation and it was suggested that 2,6-DMBQ was transformed into inactive metabolites and/or DNA repair occurred (Brambilla et al. 1988).

Sprague-Dawley rats killed 1 h following a single gavage dose of 33, 100, or 300 mg 2,6-DMBQ/kg bw showed no DNA damage in the liver, but a dose-dependent and statistically significant increase in DNA fragmentation was reported in kidney, gastric mucosa, and brain cells (Brambilla et al. 1988). Rats killed 4 or 24 h after dosing showed a partial repair of the DNA damage.

Although some weak genetic toxicity was reported with isolated 2,6-DMBQ, the concentrations and doses were 3 to 4 orders of magnitude higher than any potential ingestion exposure from Avemar pulvis (33 to 300 mg DMBQ/kg bw/day exhibiting effects versus 24.2 μg DMBQ/kg bw/day human exposure). Moreover, genetic testing with Avemar pulvis showed no evidence of genotoxic or mutagenic potential.

Special Studies

Avemar pulvis was administered by gavage at a dose of 3000 mg/kg bw/day to 8- to 10-week-old C57BL mice previously inoculated with a highly metastatic line of Lewis lung carcinoma cells (3LL-HH) (Szende et al. 2004). The purpose of this study was to investigate the effects of administering Avemar pulvis and the cytostatic drugs Endoxan, Navelbine, and doxorubicin on tumor growth and survival of animals. Thirty-five mice received a cytostatic drug plus Avemar pulvis, 30 mice received Avemar pulvis alone, 35 mice received a cytostatic drug alone, and 35 mice were controls (inoculated with 3LL-HH tumor). The animals were treated until death from the tumor inoculations, usually 18 to 60 days. All animal deaths were due to the inoculated tumors. No toxicity was seen from the administration of Avemar pulvis alone, and the combination of Avemar

pulvis with the cytostatic drugs did not increase the toxicity of the cytostatic compounds and did not exert any toxic effect.

In a study to examine the effect of Avemar pulvis alone and Avemar pulvis plus vitamin C on tumor growth and metastasis, 8- to 10-week-old inbred rats and mice had their spleens injected with tumor cells followed by gavage administration of 3000 mg Avemar pulvis/kg bw/day alone or with vitamin C (Hidvegi et al. 1998). Control animals were administered water. The animals were observed for 35, 45, and 65 days. No adverse reactions or test compound—related deaths were reported due to either Avemar pulvis alone or in combination with vitamin C.

The effect of Avemar pulvis on blastic transformation of peripheral blood lymphocytes of 8-week-old C57BL mice was investigated (Hidvegi et al. 1999). Twenty mice were administered 3000 mg Avemar pulvis/kg bw/day, 5 days/week for 6 weeks. Spleen cells were harvested from treated and control mice and then exposed to concanavalin A to cause blastic transformation. No adverse reactions or Avemar pulvis—related deaths were reported.

In order to study the ability of Avemar pulvis to prevent colon cancer in laboratory animals, F344 rats were left untreated or given three injections of azoxymethane to induce colonic carcinogenesis, followed by 3000 mg Avemar pulvis/kg bw/day administered by gavage, and observed for 32 weeks (Zalatnai et al. 2001). Thirty-two rats received the induction injections plus Avemar pulvis, 48 rats received the induction injections only, 10 rats received Avemar pulvis only, and 10 rats served as untreated controls. There were no effects on body weight and no intestinal lesions were observed in rats not administered induction injections. No other organs were affected.

CLINICAL STUDIES

In addition to the safety studies conducted in laboratory animals and in in vitro mammalian and bacterial systems, numerous published and unpublished clinical studies have been performed with Avemar pulvis and are described below (Balogh 2001a, 2001b; Demidov et al. 2002; Hidvegi et al. 2003; Jakab et al. 2003; Garami et al. 2004; Sebestyen 2004, 2005; Hoffman 2005; Balint et al. 2006). None of these studies was specifically designed to identify possible adverse effects of Avemar pulvis; rather, the studies were typically conducted to assess the beneficial effects of Avemar pulvis when used in combination with other treatments in cancer and postsurgical subjects. Although most of these studies have design flaws (e.g., small number of subjects, test and control groups not matched) or were only available in abstract form, these studies corroborate the evidence from the animal studies and the widespread use of wheat germ that use of Avemar pulvis, at the intended dose level, would not be expected to result in adverse effects. In addition, many of the study participants were in late stages of terminal cancer or otherwise weakened states during which they might be expected to be unusually sensitive to any potential toxicity of the test material.

In the majority of these studies, adults diagnosed with oral carcinoma ($n = 22$), malignant skin melanoma ($n = 19$), terminal lung cancer ($n = 17$), colorectal cancer ($n = 66$), or recuperating from breast cancer surgery ($n = 39$ and $n = 55$) were administered 8.5 g of Avemar pulvis daily in combination with chemotherapy or other drug therapies for periods averaging 7.9 to 32.2 months (Balogh 2001a, 2001b; Demidov et al. 2002; Hidvegi et al. 2003; Jakab et al. 2003). No adverse effects were reported at this dose level in any of the studies, with the exception of a few colorectal cancer subjects reporting diarrhea (4/66), nausea (2/66), and flatulence, satiation, soft stools, and constipation (1/66) (Jakab et al. 2003). In several of the studies, Avemar pulvis supplementation appeared to reduce side effects (e.g., fatigue, constipation, nausea, fever or infection, and insomnia) compared to chemotherapy alone (Balogh 2001a, 2001b; Demidov et al. 2002; Hidvegi et al. 2003; Jakab et al. 2003). In one study of 170 colorectal cancer patients (Jakab et al. 2003), not only were there no adverse effects due to adjunct therapy with Avemar pulvis, but also the survival rate was improved over those receiving standard therapy alone. In a study by Balint et al. (2006), 15 female rheumatoid arthritis patients were given twice-daily doses of 8.5 g Avemar pulvis/day totaling 17 g/day for a period of 12 months as a supplement to their steroid therapy. Treatment with Avemar pulvis allowed reduction in the doses of steroids in many patients; it was reported that no adverse effects related to Avemar pulvis were observed.

Children (11 matched pairs; aged under 18 years) with various proven solid malignant tumors were given 12 g/m^2/day (equivalent to 27 g/day for a child 1.5 m in height) of Avemar pulvis in 2 daily doses for an average of 29 months (Garami et al. 2004). No adverse effects related to treatment with Avemar pulvis were reported; moreover, Avemar pulvis appeared to reduce the incidence of treatment-related febrile neutropenia.

In three other clinical studies, hematological data were examined in cancer patients given one 8.5-g dose of Avemar pulvis daily. In one study (Sebestyen 2004), breast and colon cancer subjects were treated for 3 years, whereas in another (Sebestyen 2005) gastrointestinal (primarily colorectal), cancer patients were treated for 5 years. It was concluded from both studies that Avemar pulvis treatment had no deleterious effect on the hematological status (white blood cell count, red blood cell count, hemoglobin level, hematocrit, platelet count, erythrocyte sedimentation rate, lymphocyte count, neutrophils granulocyte count, monocyte count, eosinophil granulocyte count, and prothrombin level) of the subjects. In a third study (Hoffman 2005), subjects ($n = 8$) with various cancers (colorectal, breast, pleural, and melanoma) receiving Avemar pulvis for 3 to 6 years had prothrombin activity, coagulation potential, thromboplastin time, thrombin time, and fibrinogen production values all within normal limits (Hoffman 2005).

DISCUSSION

Avemar pulvis is a chemically well-characterized substance produced using food-grade starting materials that have a long

history of use in the United States and worldwide. In a commercial form with added flavors and sweeteners, it has been marketed over the counter in numerous countries for several years with no indication of adverse effects.

The primary basis on which the safety of Avemar pulvis is established is that it consists primarily of fermented wheat germ with the added drying aids maltodextrin and silicon dioxide. Wheat germ, both fermented and unfermented, has been a part of the human diet for millennia with no indication that it poses any health hazards. The fermented wheat germ in Avemar pulvis is substantially equivalent to the fermented wheat germ found in whole-wheat foods and it is likely that many individuals have daily intakes of wheat germ in excess of 10 g from consumption of whole wheat products. Thus, historically safe consumption of whole-wheat products provides strong evidence of the safety of Avemar pulvis for its intended use.

In addition to a history of safe use of wheat germ, the safety of Avemar pulvis has been evaluated in acute, subacute, subchronic, and genetic toxicity studies and found to have no adverse effects at exposures far in excess of those that are expected to result from its intended use. In experimental animals, Avemar pulvis has been administered at high oral doses (commonly 3 g/kg bw/day and up to 5 g/kg bw/day) for extended periods of time without any evidence of toxicity. Data from clinical studies, often with humans in late-stage disease states and having weakened resistance, using dose levels (8.5 g/day) similar to those resulting from the intended use of Avemar pulvis, further demonstrate its safety over extended periods of time. Indeed, in several instances, supplementation with Avemar pulvis appeared to lessen the side effects of drug therapy.

The available data support the conclusion that the intake of Avemar pulvis would not be expected to cause adverse effects in humans under the conditions of its intended use as an ingredient in dietary supplements to be taken at recommended doses for extended periods of time.

REFERENCES

Bahnemann, R., M. Jacobs, E. Karbe, W. Kauffmann, G. Morawietz, T. Nolte, and S. Rittinghausen. 1995. RITA (Registry of Industrial Toxicology Animal data) guides for organ sampling and trimming procedures in rats. *Exp. Toxicol. Pathol.* 47:247–266.

Balint, G., A. Apathy, M. Gaal, A. Telekes, A. Resetar, G. Blazso, G. Falkay, B. Szende, A. Paksy, M. Ehrenfeld, Y. Shoenfeld, and M. Hidvegi. 2006. Effect of Avemar—a fermented wheat germ extract—on rheumatoid arthritis. Preliminary data. *Clin. Exp. Rheumatol.* 24:325–328.

Balogh, A. 2001a. Supportive effects of Avemar in breast cancer. In: Development of biotechnologically produced—fermented—wheat germ extract (Avemar) and its use in the supportive therapy of cancer. Biotechnologia 2000. Hungarian Government Grant number OMFB-02440/2000, Budapest, Hungary. [Unpublished report.]

Balogh, A. 2001b. Effects of long-term Avemar treatment on quality of life in breast cancer. In Development of biotechnologically produced—fermented—wheat germ extract (Avemar) and its use in the supportive therapy of cancer. Biotechnologia 2000. Hungarian Government Grant number OMFB-02440/2000, Budapest, Hungary. [Unpublished report.]

Boros, L. G., M. Nichelatti, and Y. Shoenfeld. 2005. Fermented wheat germ extract (Avemar) in the treatment of cancer and autoimmune diseases. *Ann. N. Y. Acad. Sci.* 1051:529–542.

Brambilla, G., E. Cajelli, M. Mazzei, A. Pino, and L. Robbiano. 1986. Formation of genotoxic products by interaction of dimethophrine with nitrite. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 27:105.

Brambilla, G., L. Robbiano, E. Cajelli, A. Martelli, F. Turmolini, and M. Mazzei. 1988. Cytotoxic DNA-damaging and mutagenic properties of 2,6 dimethoxy-1,4-benzoquinone formed by dimethophrine-nitrite interaction. *J. Pharmacol. Exp. Therapeut.* 244:1011–1015.

Csik, M. 2000. Genotoxicology analysis of Avemar and azoxymethane in the rat's marrow micro-nucleus test. Expert's Opinion. Report number 016.00.358.MN. Department of Experimental Pathology and Mutagenic Research, National Institute of Chemical Safety, Budapest, Hungary. [Unpublished report.]

Csiko, G., G. Semjen, R. Glavits, V. Ratz, and G. Sebestyen. 2000. Subacute oral toxicity study of Avemar with 28-day treatment and 14-day post-treatment period in rat: Final report. University of Veterinary Science, Department of Pharmacology and Toxicology. Budapest, Hungary. [Unpublished report on file at Biromedicina Corporation, Budapest, Hungary.]

Csiko, G., G. Semjen, and V. Ratz. 1999. Acute oral toxicity study of Avemar in rat: Final report. University of Veterinary Science, Department of Pharmacology and Toxicology. Budapest, Hungary. [Unpublished report on file at Biromedicina Corporation, Budapest, Hungary.]

Demidov, L. V., V. Manzjuk, G. Y. Kharkevitch, N. A. Pirogova, and E. V. Artamonova. 2002. Antimetastatic effect of Avemar in high-risk melanoma patients. Interim results of an ongoing study presented at the 18th UICC International Cancer Congress, Oslo, Norway, July. Abstract No. P868. *Int. J. Cancer* 100(Suppl 13):408.

FIP (First Institute of Pathology). 1997. 77 days-long toxicity study of Avemar (M1) in F344 rats and in C57BL/6 mice. Semmelweiss Medical University. Budapest, Hungary. [Unpublished report on file at Biromedicina Corporation, Budapest, Hungary.]

Garami, M., D. Schuler, M. Babosa, G. Borgulya, P. Hauser, J. Muller, A. Paksy, E. Szabo, M. Hidvegi, and G. Fekete. 2004. Fermented wheat germ extract reduces chemotherapy-induced febrile neutropenia in pediatric cancer patients. *J. Pediatr. Hematol. Oncol.* 26:631–635.

Handa, S. S., A. D. Kinghorn, G. A. Cordell, and N. R. Farnsworth. 1983. Plant anticancer agents. XXVI. Constituents of *Peddiea fischeri*. *J. Nat. Prod.* 46:248–250.

Hidvegi, M., J. Moldvay, K. Lapis, and Z. Ajkay. 2003. Fermented wheat germ extract improves quality of life in lung cancer patients. (In Hungarian). *Medicus Anonymus/Pulmono* 11(Suppl 1):13–14.

Hidvegi, M., E. Raso, R. T. Farkas, K. Lapis, and B. Szende. 1999. Effect of MSC on the immune response of mice. *Immunopharmacology* 41:183–186.

Hidvegi, M., E. Raso, R. Tomoskozi-Farkas, S. Paku, K. Lapis, and B. Szende. 1998. Effect of Avemar and Avemar + vitamin C on tumor growth and metastasis in experimental animals. *Anticancer Res.* 18:2353–2358.

Hoffmann, A. 2005. Evaluation of the safety of long-term administration of Avemar on hemostasis in cancer patients. Budapest, Hungary. [Unpublished report on file at Biromedicina Corporation, Budapest, Hungary.]

IPCM (Institute of Preventive and Clinical Medicine). 1999. Acute oral toxicity study of Avemar in rat: Final report. Ministry of Health, Slovakia (Bratislava). [Unpublished report on file at Biromedicina Corporation, Budapest, Hungary.]

Jakab, F., Y. Shoenfeld, A. Balogh, M. Nichelatti, A. Hoffmann, Z. Kahan, K. Lapis, A. Mayer, P. Sapy, F. Szentpetery, A. Telekes, L. Thurzo, A. Vagvolgyi, and M. Hidvegi. 2003. A medical nutriment has supportive value in the treatment of colorectal cancer. *Br. J. Cancer* 89:465–469.

Lehel, J., G. Semjen, and V. Ratz. 1999. Acute oral toxicity study of Avemar in mouse: Final report. University of Veterinary Science, Department of Pharmacology and Toxicology. Budapest, Hungary. [Unpublished report on file at Biromedicina Corporation, Budapest, Hungary.]

Mohtashamipur, E., and K. Norpoth. 1984. Non-mutagenicity of some wood-related compounds in the bacterial/microsome plate incorporation and microsuspension assays. *Int. Arch. Occup. Environ. Health* 54:83–90.

Nichelatti, M., and M. Hidvegi. 2002. Experimental and clinical results with Avemar (a dried extract from fermented wheat germ) in animal cancer models and in cancer patients. *Nogyogyaszati Onkologia* 7:180–185.

NICS (National Institute of Chemical Safety). 2004. Chemo-prevention of the toxicity of nickel, and of the mutagenic toxicities of cobalt, urethane and formaldehyde in *Drosophila melanogaster* by Avemar. Fodor Jozsef National Center of Public Health. Budapest, Hungary. [Unpublished report on file at Biromedicina Corporation, Budapest, Hungary.]

Posner, E. S. 1985. The technology of wheat germ separation in flour mills. Assoc Operative Millers Bull, Suppl 1,2 (October).

Sebestyen, G. 2004. Effect of long-lasting adjuvant Avemar treatment on the hematologic status of carcinoma patients. Budapest, Hungary. [Unpublished report on file at Biromedicina Corporation, Budapest, Hungary.]

Sebestyen, G. 2005. Effect of 5-year adjuvant Avemar treatment on the hematologic status of carcinoma patients. Budapest, Hungary. [Unpublished report on file at Biromedicina Corporation, Budapest, Hungary.]

Szende, B., Z. Marcsek, Z. Kocsis, and A. Tompa. 2004. Effect of simultaneous administration of Avemar and cytostatic drugs on viability of cell cultures, growth of experimental tumors, and survival of tumor-bearing mice. *Cancer Biother. Radiopharm*. 19:343–349.

Tomoskozi-Farkas, R., and H. G. Daood. 2004. Modification of chromatographic method for the determination of benzoquinones in cereal products. *Chromatographia* 60:S227–S230.

Tsen, C. C. 1985. Amino acid composition and biological value of cereal germs. In: *Amino acid composition and biological value of cereal proteins*, ed. R. Lásztity and M. Hidvégi, 453–466. Boston: D. Reidel.

U.S. Department of Agriculture, Economic Research Service (USDA/ERS). 2005. *U.S. per capita food consumption*. Available at the USDA/ERS website at http://www.ers.usda.gov/data/foodconsumption/

Zalatnai, A., K. Lapis, B. Szende, E. Raso, A. Telekes, A. Resetar, and M. Hidvegi. 2001. Wheat germ extract inhibits experimental colon carcinogenesis in F-344 rats. *Carcinogenesis* 22:1649–1652.